REMARKS

This Amendment cancels claims 25 and 26, and amends claims 17, 22, 27 and 30. The "alternative ii)" method steps of claim 17 are supported by page 8, lines 18-29, while the binder definitions of claims 17 and 30 are taken from canceled claim 26. The ACS risk diagnosis portion of the preamble of claim 27 is supported by page 3, lines 25-26 and page 22, line 9-19. Claims 17-22, 27, 28, 30, 33 and 34 are pending.

Examiners Grun and Shibuya are thanked for the courtesies extended to the undersigned during an interview held November 12, 2009. The Examiner Interview Summary Record accurately reflects the substance of the interview.

This Amendment overcomes the objection to claim 17. More particularly, the typographical errors noted by the Patent Office has been corrected. Reconsideration and withdrawal of the objection to claim 17 are requested.

This Amendment overcomes the 35 U.S.C. § 112, second paragraph, rejection of claims 17-22, 25-28, 30, 33 and 34. More particularly, claim 17 has been amended to unambiguously define its alternative ii) by inclusion of active, positive method steps, by defining both the "first binder" and "second binder" as

(independently) an antibody or antibody fragment, and by changing "the bioaffinity reaction" to -- a bioaffinity reaction-- to overcome the lack of antecedent basis ground for rejection.

Claim 22 has been amended by replacing "used" with "detected".

Claim 25 has been canceled.

Claim 26 has been canceled.

The preamble of claim 27 has been rewritten to make clear the claimed method encompasses both diagnosing persons suffering from an acute coronary syndrome and persons at risk of acute coronary syndrome. Moreover, claim 27 has been further amended by replacing "is" with --consists of-- to make it even more clear the marker is exclusively "free" PAPP-A, expressly defined in the claim as pregnancy-associate plasma protein A which is not complexed to a proform of major basic protein (proMBP).

Claim 27 recites the positive, sequential method steps of "comparing" and "diagnosing". One of ordinary skill in the art would find nothing indefinite regarding these method steps. The applicant is entitled to claim his invention as he - not the Patent Office - sees fit. Moreover, claim breadth alone is not indefinite. See In re Gardner, 427 F.2d 786, 788, 166 USPQ 138, 140 (CCPA 1970).

During the interview, Examiner Shibuya questioned whether claim 27 was indefinite because its definition of "marker" includes unit-less ratios of compounds as well as the compounds themselves. However, the applicants are permitted to claim their invention as they see fit, as long as one of ordinary skill in the art would understand the metes and bounds of the claimed invention. In this case, the definition of "marker" in claim 27 is quite precise. In short, claim 27 is not indefinite to one of ordinary skill in the art.

Claim 30 has been amended to define the first and second binders as, independently, an antibody or antibody fragment.

Reconsideration and withdrawal of the indefiniteness rejection of claims 17-22, 25-28, 30, 33 and 34 are requested.

This Amendment overcomes the 35 U.S.C. § 112, first paragraph, rejection of claims 17-22, 25-28,30, 33 and 34 for failing to provide an adequate written description of the invention. More particularly, independent claims 17 and 27 have been amended to specify the first and second binders are, independently, an antibody or an antibody fragment[1].

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[1]The use of antibody fragments as binders is described in the application at page 7, last line through page 8, line 14. Moreover, the use of antibody fragments in immunochemistry and the production of such fragments has been very well known for about

The Examiner's objection on page 3 of the office Action, lines 8-11, saying that "the applicant does not teach an antibody that binds to PAPP-A only when complexed to proMBP" is not understood. Page 13, lines 14-15, of the application state that "Of the 17 mabs, B1, 2, 3, and 4 were previously shown to be **specific for binding to PAPP-A subunit** of the PAPP-A/proMBP complex..."

A binder which is specific for binding to PAPP-A subunit of the PAPP-A/proMBP complex will detect both free PAPP-A and PAPP-A which is complexed to proMBP. Conversely, a binder which is directed to the pro-MBP portion of the PAPP-A/proMBP complex will detect only complexed PAPP-A, and not free PAPP-A.

It is not possible to measure total PAPP-A with antibodies directed only to the proMBP epitope. Nor could the proMBP/PAPP-A complex be discriminated from non-complexed (free) PAPP-A by using only a binder directed to the PAPP-A portion of the complex.

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five decades. Related techniques have been extensively reviewed for both polyclonal [See Edelman et al., 112 J. Exp Med. 203-23 (1960); Giltlin et al., 190 Nature 634-5 (1961); Goodman, 106 Proc.Soc.Exp.Biol Med. 822-5 (1961); and Stanworth et al., Chapter 6 of 1 Handbook of Experimental Immunology. (1978)] and monoclonal antibodies (See Parham, 131 J. Immunology 2895-2902 (1983). The database www.pubmed.com gives more than 18000 hits on the search strategy "antibody and fragment". Accordingly, one of ordinary skill in the art would understand the inventors to have possession of the presently-claimed invention as of the filing date of their application.

The specification discloses 17 monoclonal antibodies which are commercially available. These 17 mabs and their epitopes are listed in the following Table:

Table: Epitope locations of mabs reactive with PAPP-A/proMBP complex

Mab	Epitope location	Reference
B1	PAPP-A subunit	Qin's PhD thesis (1998)
B2	PAPP-A subunit	Qin's PhD thesis (1998)
B3	PAPP-A subunit	Qin's PhD thesis (1998)
B4	PAPP-A subunit	Qin's PhD thesis (1998)
B5	proMBP subunit	Qin's PhD thesis (1998)
B6	proMBP subunit	Qin's PhD thesis (1998)
A1	PAPP-A subunit	WO 2005/073727 and Clin Chem 51:75-83 (2005)
A2	PAPP-A subunit	WO 2005/073727 and Clin Chem 51:75-83 (2005)
A3	PAPP-A subunit	WO 2005/073727 and Clin Chem 51:75-83 (2005)
A4	PAPP-A subunit	WO 2005/073727 and Clin Chem 51:75-83 (2005)
A5	PAPP-A subunit	WO 2005/073727 and Clin Chem 51:75-83 (2005)
A6	PAPP-A subunit	WO 2005/073727 and Clin Chem 51:75-83 (2005)
A7	PAPP-A subunit	WO 2005/073727 and Clin Chem 51:75-83 (2005)
A8	PAPP-A subunit	WO 2005/073727 and Clin Chem 51:75-83 (2005)
A9	PAPP-A subunit	WO 2005/073727 and Clin Chem 51:75-83 (2005)
A10	PAPP-A subunit	WO 2005/073727 and Clin Chem 51:75-83 (2005)
A11	proMBP subunit	WO 2005/073727 and Clin Chem 51:75-83 (2005)

Antibodies B5, B6 and A11, which are directed to epitopes on the proMBP subunit only, measure the PAPP-A/proMBP complex only. The remaining antibodies, which are directed to epitopes on the PAPP-A subunit, measure the PAPP-A/proMBP complex **and** **PAPP-A that**

is not complexed with proMBP (in other words, these antibodies measure total PAPP-A in the sample). Free PAPP-A can be calculated as a difference between total PAPP-A and PAPP-A complexed with proMBP.

The application does not disclose an example of an antibody measuring exclusively free PAPP-A in a sample comprising both free PAPP-A and proMBP-complexed PAPP-A. However, the applicants are not claiming such an antibody.

Applicants have already provided two lines of evidence, generated from gel filtration on a Superose™ 6 precision column and from two immunoassays that measure total PAPP-A and PAPP-A complexed to proMBP, respectively, which show that complexed PAPP-A (~ 700 kDa) is larger than free PAPP-A (~ 530 kDa), and furthermore, complexed PAPP-A is detected equally by the two immunoassays, whereas free PAPP-A is detected only by the assay for total PAPP-A. The combined use of the two assays enables the measurement of free PAPP-A in the sera of ACS patients.

The unavailability of an antibody which only binds to complexed PAPP-A does not affect the reliable measurement of complexed PAPP-A with the immunoassay, which is configured with one

proMBP subunit-specific antibody as tracer and one PAPP-A subunit-specific antibody as capture.

Reconsideration and withdrawal of the 35 U.S.C. § 112, first paragraph, rejection of claims 17-22, 25-28, 30, 33 and 34 are respectfully requested.

This Amendment overcomes the 35 U.S.C. § 112, first paragraph, rejection of claims 17-22 for non-enablement with respect to the PAPP-A/proMBP complex blocking embodiment. The blocking embodiment has been deleted from claim 17. Reconsideration and withdrawal of the non-enablement rejection of claims 17-22 are respectfully requested.

This Amendment overcomes the 35 U.S.C. § 102(b) rejection of claims 17 and 26 over WO 00/54806 to Overgaard et al. Claim 26 has been canceled, and claim 17 amended to more clearly define its alternative ii) which measures only free PAPP-A by a direct bioaffinity assay in which PAPP-A complexed to proMBP is first made non-capable of participating in a bioaffinity reaction. More particularly, free PAPP-A in a sample is measured by

exposing said sample to a first binder which binds to pro-MBP,

allowing said proMBP to bind to said first binder,

absorbing said first binder onto a solid phase and separating said first binder and said bound proMBP from said sample,

exposing said sample, from which proMBP has been separated, to a second binder which binds total PAPP-A, and

detecting the bound PAPP-A.

Overgaard et al. fails to disclose these steps of the claimed method. In particular, the Patent Office argument that "expression of recombinant PAPP-A in a human cell that does not produce promMBP and assay of supernatant fluid, i.e., in an person's sample" is considered to determine free PAPP-A is without merit. First, the claimed method concerns the determination of free PAPP-A in a person's sample which contains both free PAPP-A and proMBP-complexed PAPP-A, rather than a fictitious "sample" which is free from proMBP-complexed PAPP-A from the very beginning. Second, the pre-absorption step clearly distinguishes claim 17 from Overgaard et al.

Reconsideration and withdrawal of the anticipation rejection of claims 17 and 26 over WO 00/54806 to Overgaard et al. are respectfully requested.

The disclosure of U.S. Patent No. 7,115,382 to Overgaard et al. is the same as that of WO 00/54806 to Overgaard et al.

According, the 35 U.S.C. § 102(e)(2) rejection of claims 17 and 26 over U.S. Patent 7,115,382 to Overgaard et al. is traversed for the same reasons as set forth above. Reconsideration and withdrawal of the anticipation rejection of claims 17 and 26 over U.S. Patent No. 7,115,382 to Overgaard et al. are requested.

The 35 U.S.C. § 102(b) rejection of claims 27 and 28 over U.S. Patent No. 6,500,630 to Conover et al. is respectfully traversed. The claimed method requires the comparison of an amount of a marker present in a sample derived from a person to a reference amount of the marker, where the marker consists of free PAPP-A, rather than complexed PAPP-A, or the marker is a ratio based on free PAPP-A.

Before discussing Conover et al. in detail, the undersigned wishes to clear the record. Contrary to the assertion on page 9, 18-21 of the Official Action, Dr. Pettersson's Rule 132 declaration does not contain any admission against interest regarding the method disclosed in Conover et al. Similarly, the Amendments filed September 18, 2008 and April 16, 2009 do not contain any admission against interest regarding Conover et al.

Conover et al. fails to disclose the use of free PAPP-A as a marker for acute coronary syndrome for at least three reasons:

1. Conover et al. does not disclose a means for detecting exclusively free PAPP-A in a sample comprising free PAPP-A and proMBP-complexed PAPP-A

Conover et al. state that "monoclonal antibodies having specific binding affinity for PAPP-A, but not for PAPP-A/proMBP complexes, can be produced through standard methods". Applicants have previously noted this statement is subject to two alternative interpretations. Under the first interpretation[2], the monoclonal antibody will detect total PAPP-A rather than free PAPP-A (and thus not anticipate claims 27 and 28). Under the second alternative[3], the monoclonal antibody will detect only free PAPP-A.

Conover et al.'s experimental arrangement will detect total PAPP-A. See Dr. Pettersson's Rule 132 Declaration filed April 16, 2009 and the inventors' comments attached to the Amendment filed September 18, 2008. Accordingly, Conover et al.'s ambiguous

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[2]**Interpretation 1**: The monoclonal antibody (mab) has specific binding affinity for PAPP-A, but it does not have specific binding affinity for the PAPP-A/proMBP complex. This means that the mab in fact can recognized also the PAPP-A/proMBP complex although the mab is not specific for the complex. If both PAPP-A/proMBP complex and free PAPP-A are present in a sample, such a mab will detect both of them, i.e., total PAPP-A.

[3]**Interpretation 2**: The mab has specific binding affinity for PAPP-A, and the mab is not able to detect the PAPP-A/proMBP complex (i.e. the mab is directed to an epitope region of PAPP-A that would be occupied by proMBP if PAPP-A exists in PAPP-A/proMBP complex). This means the mab detects free PAPP-A only.

disclosure at Col. 4, lines 36-49 is properly interpreted to disclose the non-anticipatory alternative 1 so as to be consistent with their experimental arrangement.

2. Conover et al. fails to disclose an assay which inherently measures free PAPP-A

Conover et al. also state that PAPP-A activity can be detected by incubating the sample in a well that contains immobilized, specifically labeled substrate. Upon proteolytic cleavage of the substrate, labeled fragments are liberated into the liquid phase and detected (Col. 7, lines 38-44).

The above-summarized statement does not inherently "disclose" measurement of exclusively free PAPP-A because a protease activity assay for free PAPP-A measurement will not completely distinguish free PAPP-A from PAPP-A/proMBP complex. According to published studies[4], highly purified PAPP-A/proMBP, as described by Oxvig et al., 1201 Biochim Biophys Acta. 415-423 (1994)(of record), contains IGFBP4 proteolytic activity. This activity likely has the following two origins: first, existence of a 2:1 PAPP-A/proMBP complex allows PAPP-A proteolytic activity to be incompletely inhibited; second, the proteolytic activity of PAPP-A is not

[4]See Lawrence et al., 96 Proc Natl Acad Sci U S A 963149-53 (1999); and Overgaarde et al., 275 J. Biol Chem. 31128-33 (2000); both of record.

completely inhibited by proMBP even for a 2:2 PAPP-A/proMBP complex. Thus, a protease activity assay for measurement of free PAPP-A has a fundamental problem in specificity because it is incapable of completely distinguishing free PAPP-A from PAPP-A/proMBP complex.

Moreover, "PAPP-A activity" theoretically relates to *any* form of PAPP-A which is enzymatically active. Uncomplexed intact PAPP-A is expected to be enzymatically active, but it is generally known that many proteases while appearing in a free form may still lack enzymatic activity due to having internal cleavages or representing a proform of the protease. See Chua et al., 275 J.Biol.Chem. 5131-5 (2000); Borgono et al., 2 Mol. Cancer Res. 257-80 (2004); and Wu et al., 58 Prostate 345-53 (2004), all of record.

The determination of activity relies heavily on the specificity of the substrate used. Immunoassays can be designed to measure free PAPP-A regardless whether it is proteolytically active or not. In contrast, protease assays only determine proteolytically active forms. In case PAPP-A is partially complexed with proMBP, meaning that only one proMBP subunit is complexed with two PAPP-A subunits [see Overgaard et al., 275 J. Biol Chem. 31128-33 (2000)(of record)], a protease assay can give misleading results, while an immunoassay will not.

It is important to note that methods for detecting an ACS-related increase in free PAPP-A must be extremely sensitive and rapid due to the acute nature of the disease. Enzyme activity assays can satisfy neither of these requirements. Instead, enzymatic assays are known to be inherently less sensitive than immunoassays employing a non-competitive (sandwich) assay design with a reporter system of high specific activity. The inventors are unaware of any clinical data which proves the value of activity assays for patients at risk for (or suffering from) acute cardiac syndrome.

3. Conover et al. Failed to Analyze for Exclusively Free PAPP-A

The Patent Office cites Col. 6, lines 25-30 to show an antibody useful for binding exclusively free PAPP-A and an approach for the production of such an antibody. However, the applicants are not claiming an antibody per se or a means for producing an antibody useful for exclusively binding free PAPP-A. Instead, claims 27 and 28 recite a method for diagnosing persons suffering from an acute coronary syndrome and persons at risk of acute coronary syndrome, using exclusively free PAPP-A as a marker.

The mere suggestion or disclosure of an antibody specific for uncomplexed PAPP-A and an assay for measuring uncomplexed PAPP-A do

not disclose the claimed method for diagnosing persons suffering from an acute coronary syndrome and persons at risk of acute coronary syndrome using exclusively free PAPP-A as a marker.

If Conover et al. had realized that exclusively free PAPP-A is a better marker than total PAPP-A, they could have determined exclusively free PAPP-A as "total PAPP-A" minus "proMBP-complexed PAPP-A" using antibodies which were available on their application date[5]. Conover et al.'s failure to analyze for exclusively free PAPP-A proves they did not realize that exclusively free PAPP-A is a better marker for ACS diagnosis.

Col. 6, lines 25-30 of Conover et al. merely suggest an idea of how to generate antibodies exclusively reactive with free PAPP-A. However, Conover et al. fails to measure exclusively free PAPP-A, and fails to suggest that exclusively free PAPP-A would be a good marker for diagnosing ACS. This failure proves that they did not realize that exclusively free PAPP-A would be a better marker for ACS diagnosis. They had not invented the use of exclusively free PAPP-A as marker for diagnosing persons suffering from an

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[5] Mabs 234-2, 234-3, 234-4, 234-5 and 234-6 measure total PAPP-A, while mabs 234-8 and 234-10 detect proMBP; for 234-8 and 234-10 see Article 2 of the thesis by Qin, page 5, paragraph 3.2 and Table 1.

acute coronary syndrome and persons at risk of acute coronary syndrome diagnosing an acute coronary syndrome.

Reconsideration and withdrawal of the anticipation rejection of claims 27 and 28 over Conover et al. are requested.

A Rule 132 declaration regarding inventorship of the subject matter disclosed in an electronic publication is attached.

It is believed this application is in condition for allowance. Reconsideration and withdrawal of all rejections of claims 17-22, 25-28, 30, 33 and 34, and issuance of a Notice of Allowance directed to claims 17-22, 27, 28, 30, 33 and 34, are respectfully requested. The Examiner is urged to telephone the undersigned should he believe any further action is required for allowance.

The fee for the extension of time is being paid electronically today. It is not believed any additional fee is required for entry and consideration of this Amendment. Nevertheless, the Commissioner

is authorized to charge Deposit Account No. 50-1258 in the amount of any such required fee.

Respectfully submitted,

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Enclosures:
Petition for Extension of Time
Rule 132 Declaration